इंटरनेट मानक

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"पुराने को छोड नये के तरफ"
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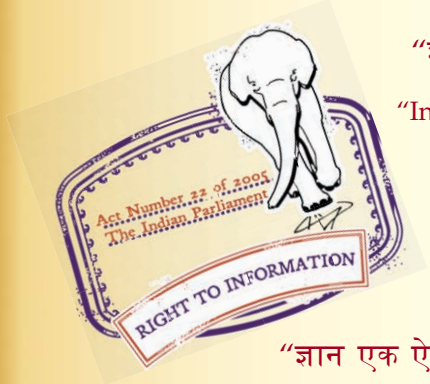
IS 10972 (1984): Code for preparation of ESCHERICHIA COLI diagnostic sera [FAD 15: Food Hygiene, Safety Management and Other Systems]



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Bhartṛhari—Nītiśatakam
"Knowledge is such a treasure which cannot be stolen"

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IS : 10972 - 1984
(Reaffirmed 2005)

Indian Standard

CODE FOR PREPARATION OF *ESCHERICHIA COLI* DIAGNOSTIC SERA

UDC 616·981·48-07 : 616·15-078-097·3 : 613·6

INDIAN STANDARDS INSTITUTION
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

March 1985

Gr 4

Indian Standard

CODE FOR PREPARATION OF *ESCHERICHIA COLI* DIAGNOSTIC SERA

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(*Continued on page* 15)

Indian Standard

CODE FOR PREPARATION OF *ESCHERICHIA COLI* DIAGNOSTIC SERA

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 30 April 1984, after the draft finalized by the Food Hygiene Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Three types of antigens, that is, Somatic (*O*-antigens), Capsular (*K*-antigens) and flagellar (*H*-antigens) are known to be present in *Escherichia coli.* So, for complete serotyping, identification of all the antigenic components is necessary. About 164 different types of *O*-antigens, 103 *K*-antigens and 56 *H*-antigens are known in *E. coli.* As such 164 *O* sera, 103 *K* sera and 56 *H* sera are required to be raised, absorbed and finally tested for any cross reactions, before attempting to serotype *E. coli* strains. To avoid confusion as a result of cross reactions, it is appropriate to pay close attention to the minute details at all the stages in the process. The sera must be produced *ONLY IN RABBITS* and in no other animal.

0.3 In the preparation of this standard, considerable assistance has been derived from the Central Research Institute Kasauli.

0.4 In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

1. SCOPE

1.1 This standard specifies the method for raising, absorption and testing of various sera to be used for serotyping of various strains of *Escherichia coli.*

2. SELECTION OF IMMUNIZING STRAINS

2.1 Strains used for the preparation of *O*, *K* and *H* sera should have been thoroughly tested for their stable antigenic value and should give

*Rules for rounding off numerical values (*revised*).

minimum possible cross reactions. It is, therefore, advisable to use standard recommended strains which have been tested over years of experimentation.

2.1.1 *Selection of* O-*Immunizing Strains* — The International *E. coli* Centre established at the Statens Serum Institute, Copenhagen, Denmark has recommended 164 strains for preparation of *O*-immunizing suspensions as shown in Appendix A.

2.1.2 *Selection of* K-*Immunizing Strains* — The International *E. coli* Centre has recommended 103 strains for the preparation of *K*-immunizing suspensions as shown in Appendix B.

2.1.3 *Selection of* H-*Immunizing Strains* — 56 standard *E. coli H* antigen strains have been recommended by the International Centre as shown in Appendix C.

2.2 The standard recommended strains of *Escherichia coli* can be obtained from the National *Salmonella* and *Escherichia* Centre and the National Collection of Type Cultures located at the Central Research Institute, Kasauli.

3. REAGENTS

3.1 Buffered Formol Saline (BFS)

a) Stock BFS 2·5 percent

Commercial formalin (40 percent)	50 ml
Physiological saline	2 000 ml

Adjust *p*H to 7·6 by addition of Na_2HPO_4

b) Working BFS 0·25 percent

Stock (2·5 percent) BFS	40 ml
Physiological Saline	360 ml

3.2 Mercuric Iodide Stock Solution (SMIS)

Mercuric iodide	1 g
Potassium iodide	4 g
Distilled water	100 ml

3.3 Bridges Solution

Stock 2·5 percent buffered formol saline (BFS)	2·5 ml
Stock mercuric iodide solution (SMIS)	10 ml
Physiological saline	90 ml

3.4 Acriflavine Solution

Make a 1 : 500 solution in distilled water

3.5 0·4 percent Phenol Saline

Make from a stock (5 percent) solution of pure phenol.

3.6 Reference Homologous Sera — These are prepared by immunizing rabbits with standard immunizing suspensions and are absorbed to make them monospecific. These should have sufficient titre (not less than 1:160) for Agglutination tests.

3.7 Formalized Broth Culture — To an 18 to 20 hour broth culture 0·25 percent of formaline (*v/v*) is added.

4. PREPARATION OF IMMUNIZING SUSPENSION

4.1 *O*. Suspension — The *O* antigens of *E. coli* are heat stable and are not inactivated by heating at 100°C for 2 hours 30 minutes.

4.1.1 Before attempting to prepare *O*. suspension, one must be sure that the strain is not rough and does not show autoagglutination. The recommended *O* antigen strain is subcultured on nutrient agar [*see* IS : 5887 (Part 3) - 1976*] from a 4 hour nutrient broth culture [*see* IS : 5887 (Part 3) 1976*] and incubated at 37°C overnight.

4.1.2 Next morning 5 smooth colonies are selected, tested for auto-agglutination in physiological saline and 1 : 500 acriflavin solution.

4.1.3 The above 5 colonies are simultaneously subcultured in 10 ml of nutrient broth and incubated at 37°C for 4 to 6 hours.

4.1.4 Inoculate a well dried agar slope and incubate at 37°C for 18 hours.

4.1.5 Harvest the growth with a sterile bent Pasteur pipette in 1 to 2 ml of physiological saline and homogenize.

4.1.6 The above suspension in sealed ampoules is subjected to heating at 100°C for 2 hours and 30 minutes and then allowed to cool.

4.1.7 The opacity of the above suspension is adjusted with 0·25 percent buffered formol saline so that it contains $2\,000 \times 10^6$ organisms per ml.

4.1.8 The immunizing suspension should be stored at +4°C.

4.2 *K*. Suspension

*Methods for detection of bacteria responsible for food poisoning : Part 3 Isolation and identification of *Salmonella* and *Shigella*.

4.2.1 Inoculate a nutrient agar slope from 4 to 6 hours nutrient broth culture as in **4.1.1.**

4.2.2 Run 2 ml of 10 percent mercuric iodide solution on the slope so as to cover the slope. Allow to stand at 25°C for 2 to 3 hours for bactericidal action. Harvest the suspension in a sterile tube and adjust the opacity to $8\,000 \times 10^6$ organisms per ml with Bridge's solution.

4.3 *H*-Suspension — Before preparing *H*-suspension, the standard strains should be given 2 to 3 blind passages in motility test medium [IS : 5887 (Part 3) - 1976*] to increase motility. At the end of the last passage, the culture should be examined by hanging drop method. For this purpose, a 2 to 3 hour old growth in nutrient broth is examined microscopically by placing a drop of this growth on a cover slip which in turn is placed in inverted position in a depression glass slide. Not less than 80 percent of bacteria should be motile. Inoculate 100 ml of nutrient broth from this culture and incubate at 37°C for 6 to 8 hours. The growth is then diluted with 0·25 percent buffered formol saline to an opacity of 400×10^6 organisms per ml using reference opacity tubes.

4.4 Testing of Immunizing Suspensions

a) The immunizing suspension should be tested for roughness as in **4.1.2.**

b) The immunizing suspension should be tested with a reference homologous sera (*see* Appendix D). It must agglutinate the homologous sera to the extent of the stated titre of the serum.

c) The immunizing suspension should be tested for sterility.

5. IMMUNIZING AND BLEEDING SCHEDULES

5.1 Selection of Rabbits — Healthy rabbits weighing about 1·5 to 2 kg are taken.

5.2 Immunization — Healthy rabbits are intravenously injected with the immunizing suspension, as per the schedule shown below :

Day	*Suspension in* ml
First	0·25
Third	0·5
Sixth	1·0
Ninth	1·5
Twelfth	2·0
Sixteenth	Bleeding

*Methods for detection of bacteria responsible for food poisoning : Part 3 Isolation and identification of *Salmonella* and *Shigella.*

5.2.1 The rabbits should be examined carefully for any side reaction. Should any reactions occur, further immunization may be discontinued.

5.3 Bleeding — Four to five days after the last injection the animal is test bled and tested for homologous titres by agglutination test (*see* Appendix D). If satisfactory, the animal is bled from the heart (20 ml) followed by another 20 ml heart bleeding 2 to 3 days later. The rabbit is finally bled by exsanguination 2 to 5 days after the second bleeding.

5.4 Blood is collected in dry sterile test tubes. The tubes are kept at 37°C for 2 hours and then in a refrigerator (+4°C) overnight. The clot is removed by centrifugation and all the sera from different bleedings pooled together. Sera showing haemolysis must be discarded.

5.5 The sera should be stored at + 4°C.

6. PRELIMINARY TESTING OF SERA

6.1 Both homologous and heterologous antibody titre of the serum may be determined by tube agglutination method (*see* Appendix D). This information may serve as a guideline in choosing strains required for absorption.

7. ABSORPTION

7.1 The absorbing strains (as determined in **6**) should be tested for smoothness as in **4.1.2**. For absorption of *O*, *H* and *K* agglutinins, the absorbing suspensions may be prepared as detailed in **4.1**, **4.2**, and **4.3**.

7.2 For absorption of serum for *O*-agglutinins approximately one 18 to 20 hours old nutrient agar slope (150×16 mm) per ml of the serum is taken. In case of absorption of *H*-agglutinins centrifuged deposit of an 18 to 20 hours formalized 10 ml broth culture tube per ml of antiserum is taken. (*see* **3.7**). For *K*-agglutinin absorption, one 18 to 20 hour nutrient agar slope per ml of serum is sufficient. When the number of strains to be absorbed are more than one, the serum is absorbed in one sitting with all the suspensions prepared from these strains. The suspension(s) (*see* **7.1**) and the serum are thoroughly mixed and kept in a water bath at 52°C for 2 hours after which the mixture is centrifuged and the serum separated.

7.3 The agglutination test against homologous and heterologous (absorbing strains) antigens is again put up. In case the heterologous antibodies still persist absorption may be repeated again by taking varied amount of bacterial mass depending on the titre of antibody to be absorbed. Further absorption after the second one is not recommended. For satisfactory results the final homologous titre should not be less than 1 : 160 and the heterologous titre 20 negative. The serum is then

seitz filtered and preserved by adding 0·4 percent phenol. It is always advisable to absorb small amounts of serum at one time as unabsorbed sera retain their titre for a longer period of time.

8. FINAL TESTING

8.1 The absorbed serum when tested finally should give the following titre.

8.1.1 *O-serum* — Homologus titre : not less then 1 : 160 but if more, to be suitably diluted to 1 : 160

Poly *H* antibody titre = less than 20
Poly *K* antibody titre = less than 20

8.1.2 *H-serum* — Homologous titre : not less than 1 : 160 but if more, to be suitably diluted to 1 : 160

Poly *O* antibody titre : less than 20
Poly *K* antibody titre : less than 20

8.1.3 *K-serum* — Homologous titre : not less than 1 : 160 but if more, to be suitably diluted to 1 : 160

Poly *O* antibody titre : less than 20
Poly *H* antibody titre : less than 20

9. TEST FOR STERILITY

9.1 The sera should be suitably tested for sterility.

10. PRESERVATION AND STORAGE

10.1 The serum should be preserved with 0·4 percent phenol and stored at +4°C. It shall never be frozen.

10.1.1 It may be distributed in aliquots of 1 ml each.

11. LABELLING

11.1 The serum should be labelled properly indicating clearly :

a) Name of the manufacturer and manufacturing licence number, if any;
b) Name of the serum shall be written as *E. coli* serum indicating clearly *O*, *H* or *K* type number;
c) Quantity, titre of the serum, the preservative used, if any and the temperature of storage;

d) Batch/lot No. and expiry date; and

e) Label should have the words 'FOR LABORATORY USE ONLY'.

12. MAINTENANCE OF RECORDS

12.1 The manufacturer should maintain proper production records right from the first stage of preparation to the last giving separate batch numbers to each lot of serum prepared. The record shall also include the various tests done on these sera during all stages including tests on sterility.

APPENDIX A

(*Clause* 2.1.1)

STANDARD *O* IMMUNIZING STRAINS

O-Antigen	*Culture No.*	*O-Antigen*	*Culture No.*
1	U5 - 41	24	E 41a
2	U9 - 41	25	E 47a
3	U14 - 41	26	H 311b
4	U4 - 41	27	F 9884
5	U1 - 41	28	K 1a
6	Bi 7458 - 41	29	Su 4338 - 41
7	Bi 7509 - 41	30	P 2a
8	G 3404 - 41	32	P 6a
9	Bi 316 - 42	33	E 40
10	Bi 8337 - 41	34	H 304
11	Bi 623 - 42	35	E 77a
12	Bi 626 - 42	36	H 502a
13	Su 4321 - 41	37	H 510c
14	Su 4411 - 41	38	F 11 621 - 41
15	F 7902 - 41	39	H 7
16	F11119 - 41	40	H 316
17	K 12 a	41	H 710c
18	F 10C 18 - 81	42	P 11a
19 a	F 8858 - 41	43	Bi 7455 - 41
19 b	F 8 188 - 41	44	H 702 C
20	P 7a	45	H 61
21	E 19a	46	P 1 C
22	E 14a	48	U8 - 41
23	E 39a	49	U 12 - 41

(*Continued*)

STANDARD *O* IMMUNIZING STRAINS

O-Antigen	*Culture No.*	*O-Antigen*	*Culture No.*
50	U 18 - 41	92	H 308a
51	U 19 - 41	93	H 308b
52	U 20 - 41	95	H 311a
53	Bi 7327 - 41	96	H 319
54	Bi 3972 - 41	97	H 320a
55	Su 3912 - 41	98	H 510d
56	Su 3684 - 41	99	H 504c
57	F 8198 - 41	100	H 509a
58	F 8962 - 41	101	H 501a
59	F 9095 - 41	102	H 511
60	F 10167a - 41	103	R 515d
61	F 10167b - 41	104	H 519
62	F 10524 - 41	105	H 520b
63	F 10598 - 41	106	H 521a
64	K 6b	107	H 705
65	K 11a	108	H 708b
66	P 6a	109	H 709c
68	P 7d	110	H 711c
69	P 9b	111	Stock W
70	P 9c	112	1411 - 50
71	P 10a	113	6182 - 50
73	P 12a	114	W 26
74	E 3a	115	W 27
75	E 3b	116	W 28
76	E 5d	117	W 30
77	E 10	118	W 31
78	E 38	119	W 34
79	E 49	120	W 35
80	E 71	121	W 39
81	H 5	123	W 43
82	H 14	124	227
83	H 17a	125	Canioni (2745 - 53)
84	H 19	126	E 611 (6021 - 50)
85	H 23	127	Holcomb (4932-53)
86	H 35	128	Cigleris (56-54)
87	H 40	129	178 - 54 (1986 - 54)
88	H 53	130	4866 - 53
89	H 68	131	HW 27
90	H 77	132	HW 30
91	H 307b	133	HW 31

(*Continued*)

STANDARD *O* IMMUNIZING STRAINS

O-Antigen	*Culture No.*	*O-Antigen*	*Culture No.*
134	4370 - 53	150	(C 853 - 66) 1935
135	Coli pecs	151	880 - 67
136	111 - 55	152	1184 - 68
137	RVC 1787	153	14097
138	62 - 57	154	E 1541 - 68
139	63 - 57	155	E 1529 - 68
140	149 - 51	156	E 1585 - 68
141	RVC 2907	157	A - 2
142	C 771	158	E 1020 - 72
143	4608 - 58	159	E 2476 - 72
144	1624 - 56	160	E 110 - 69
145	E 1385 (3)	161	E 223 - 69
146	2950 - 4A	162	10 B1/1
147	G 1253 E	163	Sn 3B/1
148	E 519 - 66	164	DRL 145/46
149	Abbot-stown A1		

APPENDIX B

(*Clause* 2.1.2)

STANDARD *K* IMMUNIZING STRAINS

K-Antigen	*Culture No.*	*K-Antigen*	*Culture No.*
1	U 9 - 41	16	K 12a
2	U 14 - 41	17	P 7a
3	U 4 - 41	18	E 39a
4	U 1 - 41	19	E 47a
5	Bi 8337 - 41	20	E 19a
6	Bi 7457 - 41	21	H 38
7	Pus 3432 - 41	22	H 67
8	G 3404 - 41	23	H 54
9	Bi 316 - 42	24	H 45
10	6233 - 42	25	Bi 7575 - 41
11	Su 4231 - 41	26	Bi 449 - 42
12	Su 65 - 42	27	E 56 B
13	Su 4344 - 41	28	K 14a
14	F 7902 - 41	29	Bi 161 - 42
15	F 8316 - 41	30	E 69

(*Continued*)

STANDARD *K* IMMUNIZING STRAINS

K-Antigen	*Culture No.*	*K-Antigen*	*Culture No.*
31	Su 3973 - 41	67	56 - 54
32	H 36	68	1411 - 50
33	Ap 189	69	34 W
34	E 75	70	2745 - 53
35	A 141a	71	E 611
36	A 198a	72	227
37	A 84a	73	909 - 51
38	A 262a	74	3354 - 53
39	A 121a	75	6182 - 50
40	A 51d	76	F 10018 - 41
41	A 433a	77	F 3219 - 54
42	A 2956	78	1111 - 55
43	A 195a	79	RCV 1787
44	A 168a	80	E 38
45	A 169a	81	62 - 57
46	A 236a	82	63 - 57
47	A 282a	83	134 - 51
48	A 290a	84	2292 - 55
49	A 180a	85	RCV 2907
50	PA 80c	86	C 771
51	A 183a	87	CG 9
52	A 103	88	E 68
53	PA 236	89	D 357
54	A 12b	90	K 10
55	N 24c	92	6181 - 66
56	H 17b	94	E 1541 - 68
57	H 909d	95	E 3b
58	Stock W	96	E 10
59	5624 - 50	97	H 5
60	E 893	98	H 705
61	E 990	99	B 41
62	S 1961	100	F 147
63	4932 - 53	101	1 413
64	5017 - 53	102	6 CB 10/1
65	2160 - 53	103	3 CE 275/6
66	1685		

APPENDIX C

(*Clause* 2.1.3)

STANDARD *H* IMMUNIZING STRAINS

H-Antigen	*Culture No.*	*H-Antigen*	*Culture No.*
1.	Su 1242	31.	HW 33
2.	Bi 7455 - 41	32.	HW 34
3.	Bu 7327 - 41	33.	HW 35
4.	U9 - 41	34.	BP 12665
5.	U4 - 41	35.	4370 - 53
6.	A 20a	36.	5017 - 53
7.	U5 - 41	37.	P 11a
8.	AP 320c	38.	P 9b
9.	Bi 7575 - 41	39.	E 3a
10.	Bi 623 - 42	40.	E 49
11.	Su 4321 - 41	41.	RVC 1787
12.	Bi 316 - 42	42.	P 9c
14.	F 10018 - 41	43.	149 - 51
15.	E 39a	44.	781 - 55
16.	8316 - 41	45.	4106 - 54
17.	P 12b	46.	5306 - 56
18.	K 12a	47.	1755 - 58
19.	A 18d	48.	P 4
20.	H 3306	49.	2147 - 59
21.	Ulla - 44	50.	6226 - 60
23.	HW 23	51.	669 - 58
24.	HW 25	52.	C 2187 - 69
25.	HW 26	53.	E 480 - 68
26.	HW 27	54.	E 223 - 69
27.	HW 28	55.	E 2987 - 73
28.	HW 30	56.	SN 3N/1
29.	HW 31		
30.	HW 32		

APPENDIX D

(*Clause* 4.4)

IMMUNIZING SUSPENSION

D-1. AGGLUTINATION TESTS

D-1.1 Serial two-fold dilutions of the test serum are made in physiological saline and 0·3 ml of each dilution (from 1:10 to 1:2560) is pipetted into different Dreyer's tubes. To each tube 0·3 ml of standard antigen is then added. A control tube containing 0·3 ml of physiological saline and the standard antigen is also included. All the tubes are placed in a water bath for 4 hours at 52°C and then at room temperature for overnight. The last dilution which shows agglutination gives the titre of the antiserum. The control tube should not show any agglutination.

(*Continued from page* 2)

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INTERNATIONAL SYSTEM OF UNITS (SI UNITS)

Base Units

Quantity	Unit	Symbol
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Thermodynamic temperature	kelvin	K
Luminous intensity	candela	cd
Amount of substance	mole	mol

Supplementary Units

Quantity	Unit	Symbol
Plane angle	radian	rad
Solid angle	steradian	sr

Derived Units

Quantity	Unit	Symbol	Definition
Force	newton	N	1 N = 1 kg.m/s^2
Energy	joule	J	1 J = 1 N.m
Power	watt	W	1 W = 1 J/s
Flux	weber	Wb	1 Wb = 1 V.s
Flux density	tesla	T	1 T = 1 Wb/m^2
Frequency	hertz	Hz	1 Hz = 1 c/s (s^{-1})
Electric conductance	siemens	S	1 S = 1 A/V
Electromotive force	volt	V	1 V = 1 W/A
Pressure, stress	pascal	Pa	1 Pa = 1 N/m^2